DATA EVALUATION RECORD

EPA Primary Reviewer: Ibrahim S. Barsoum, Ph.D. ISB

EPA Secondary Reviewer: John L. Kough, Ph.D. JLK

Acute oral toxicity

Data Record	Reg. No. 89615-R Submission No. 935475 / Decision No. 478892 / DP Barcode: DP 418701
Title & Author:	Bacillus subtilis IAB/BS/03 Technical Powder. EVALUATION OF ACUTE ORAL TOXICITY IN RATS, RICHEUX, F (2011)
MRID No:	489693-04
Guidelines:	EPA guideline 870.1100
GLP	Yes

Executive Summary

The test item *Bacillus subtilis* IAB/BS/03 Technical Powder was administered to a group of 6 female Sprague Dawley rats at the single dose of 2000 mg/kg body weight (mean : 7.8 x 10^9 CFU/animal). No mortality occurred during the study. No clinical signs related to the administration of the test item were observed. The body weight evolution of the animals remained normal throughout the study. The macroscopical examination of the animals at the end of the study did not reveal treatment related change. In conclusion, the LD_{50} of the test item *Bacillus subtilis* IAB/BS/03 Technical Powder is higher than 2000 mg/kg body weight by oral route in the rat.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** *Bacillus subtilis* IAB/BS/03 Technical powder
   Description: White powder
   Lot/Batch #: UBBS1101011
   Concentration: 2 x 10^{10} CFU/g
   Stability of test compound: Stable at room temperature.

2. **Test animals**
   Species: Rats (6)
   Sex: Female
   Strain: Sprague-Dawley (SPF Caw)
   Age: 8 weeks old
   Weight at dosing: 188 - 206 g
   Source: Elevage JANVIER (53940 Le Genest St. Isle – France)
   Acclimatisation period: 5 days prior to commencement of the study.
   Diet: Drinking water (tap-water from public distribution system) and foodstuff (M20-SDS) were supplied freely.

4. **Environmental conditions**
   Temperature: 19 - 25 °C
   Humidity: 30-70% relative humidity
   Photoperiod: Alternating 12-hour light and dark cycles
   Air rate: fifteen changes per hour

B. STUDY DESIGN AND METHODS

1. In life dates: 31st May 2011 to 15th June 2011

2. Dose and administration mode

The animals of the treated group received an effective dose of 2000 mg/kg body weight of the test item. 2 g of the test item was weighed and distilled water was added to a 10 mL volumetric flask. The preparation was magnetically stirred to obtain a brown solution just before the administration and was administered under a volume of 10 mL/kg body weight using a suitable syringe graduated fitted with an oesophageal metal canula.

II. RESULTS AND DISCUSSION

A. Mortality

No mortality occurred during the study.

B. Clinical signs

No clinical signs related to the administration of the test item were observed.

The body weight evolution of the animals remained normal throughout the study.

The macroscopical examination of the animals at the end of the study did not reveal treatment related change.

III. CONCLUSIONS

The LD50 of the test item *Bacillus subtilis I*AB/BS/03 Technical Powder is higher than 2000 mg/kg body weight by oral route in the rat.

EPA TOXICITY CATEGORY III

DATA EVALUATION RECORD

EPA Primary Reviewer: Ibrahim S. Barsoum, Ph.D. ISB

EPA Secondary Reviewer: John L. Kough, Ph.D. JLK

Acute Dermal Toxicity

Data Record	Reg. No. 89615-R Submission No. 935475 / Decision No. 478892 / DP Barcode: DP 418701 Study number TAD-PH-11/0245
Title & Author:	Bacillus subtilis IAB/BS/03 Technical Powder. EVALUATION OF ACUTE DERMAL TOXICITY IN RATS. RICHEUX, F. (2011)
MRID No:	489693-05
Guidelines:	EPA guideline 870.1200
GLP	Yes

Executive Summary

The test item *Bacillus subtilis* IAB/BS/03 Technical Powder was applied onto the intact skin of 10 Sprague Dawley rats (5 males and 5 females) at the single dose of 2000 mg/kg body weight (mean : 8.8 10^9 CFU/animal). No mortality occurred during the study. Neither cutaneous reactions nor systemic clinical signs related to the administration of the test item were observed. A slight brown coloration was noted on the treatment site of all animals on days 1 and 2. The body weight evolution of the animals remained normal throughout the study. The macroscopical examination of the animals at the end of the study did not reveal treatment-related changes. In conclusion, the LD_{50} of the test item *Bacillus subtilis* IAB/BS/03 Technical Powder is higher than 2000 mg/kg body weight by dermal route in the rat.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** *Bacillus subtilis* IAB/BS/03 Technical powder
   Description: White powder
   Lot/Batch #: UBBS1101011
   Concentration: 2×10^{10} CFU/g
   Stability of test compound: Stable at room temperature.

2. **Test animals**
   Species: Rats (10)
   Sex: Male (5) & Female (5)
   Strain: Sprague-Dawley
   Age: 7 weeks old (males); 8 weeks old (females)
   Weight at dosing: 216 - 245 g (males); 203 - 220 g (females)
   Source: Elevage JANVIER (53940 Le Genest St Isle – France)
   Acclimatisation period: 5 days prior to commencement of the study.
   Diet: Drinking water (tap-water from public distribution system) and foodstuff (M20-SDS) were supplied freely.

4. **Environmental conditions**
   Temperature: 19 - 25 °C
   Humidity: 30-70% relative humidity
   Photoperiod: Alternating 12-hour light and dark cycles

Air rate: fifteen changes per hour

B. STUDY DESIGN AND METHODS

1. **In life dates:** 31st May 2011 to 14th June 2011

2. **Dose and administration mode**

Approximately 24 hours before the treatment, fur was removed from the dorsal area of the trunk of the test animals by clipping. At least 10 per cent of the body surface area was clear for the application of the test item.

Animals from treated group received by topical application, under porous gauze dressing, an effective dose of 2000 mg/kg body weight of *Bacillus subtilis* IAB/BS/03 Technical Powder:

Four grams of the test item was weighed and distilled water was added to a 20 mL volumetric flask. The preparation was magnetically stirred to obtain a brown solution just before the administration and was administered under a volume of 10 mL/kg body weight, during 24 hours. After 24-hour exposure period, the gauze dressings were removed.

II. RESULTS AND DISCUSSION

A. Mortality

No mortality occurred during the study.

B. Clinical signs

Neither cutaneous reactions nor systemic clinical signs related to the administration of the test item were observed. A slight brown coloration was noted on the treatment site of all animals on days 1 and 2.

The body weight evolution of the animals remained normal throughout the study.

The macroscopical examination of the animals at the end of the study did not reveal treatment-related changes.

III. CONCLUSIONS

The LD_{50} of the test item *Bacillus subtilis* IAB/BS/03 Technical Powder is higher than 2000 mg/kg body weight by dermal route in the rat.

EPA TOXICITY CATEGORY III

DATA EVALUATION RECORD

EPA Primary Reviewer: Ibrahim S. Barsoum, Ph.D. ISB

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Acute inhalation toxicity

Data Record	Reg. No. 89615-R Submission No. 935475 / Decision No. 478892 / DP Barcode: DP 418701 Study Number 1358-TI
Title:	Acute inhalation toxicity.
MRID No:	489693-17
Guidelines:	EPA guideline 885.315
GLP	Yes

Executive Summary

This study was performed on behalf of IAB, S.L., trying to assess the toxic potential of a substance by single inhalation dose, and estimate the lethal concentration (LC_{50}) in rats at a level of concentration of 4.7 mg/L in air during 4 hours of *Bacillus subtilis* IABBS03. The test substance was administrated by a system of resuspension of dust which provided the material at a concentration of 4.7 mg/L of air. Ten rats (5 for each sex) of the Spragüe-Dawley strain were exposed to the product within an exposure chamber for a period of 4 hours. The results showed a mortality of 0% and calculate an CL_{50} of level upper of 4.7 mg/L in air during 4 hours, according to the recommendations of the Guide OECD N° 403. The clinical observations were conducted daily, looking for signs of toxicity or mortality in the population. Body weight was recorded weekly since the beginning of the study. At the end, the surviving animals were submitted to necropsy analysis looking for differences with the control of 5 animals from each sex. The animals exposed by inhalation route to 4.7 mg/L of the product *Bacillus subtilis* IABBS03 in air during 4 hours showed no symptoms associated with exposure of the product during the period of observation. The macroscopic pathology examination of the animals exposed to the test substance showed moderate pulmonary congestion, mild petechiae in lung and moderate to severe hepatic congestion. All these signs are consistent with breathing a microorganism dust. All the animals showed a weight increase during the period of the study. This increase was 29.5% in male and 8.5% in female. This study estimated that CL_{50} for the product *Bacillus subtilis* IABBS03 in rats is higher than 4.7 mg/L in air during 4 hours.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** *Bacillus subtilis* IAB/BS/03 Technical Powder
   Description: White solid with a characteristic odour
   Lot/Batch #: BS81101011
   Concentration: 10 g/kg (1.00×10^8 CFU/g)
   Stability of test compound: Stable at room temperature (20-25 °C). In a cool and dry place.
2. **Test animals**
   Species: Rats (10)
   Sex: Male (5) & Female (5)
   Strain: Sprague-Dawley
   Age: 8-10 weeks old
   Weight at dosing: not stated in the report
   Source: Faculty of Chemical and Pharmaceutical Sciences, University of Chile
   Acclimatisation period: 5 days prior to commencement of the study.

Diet: Water (drinking, normal) and balanced food in pellets (Champion ®), *ad libitum*.

4. Environmental conditions

Temperature: 22 ± 3 °C

Humidity: 30-70% relative humidity

Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In life dates: 17th February 2012 to 02nd March 2012

2. Preparation of the animals

Food was withdrawn from all the animals selected for the study the night before the beginning of the study (approximately 12 hours).

3. Preparation of the test substance

The test substance was passed through a 45 µm sieve size grid, and then was resuspended. The system of resuspension is a sealed chamber in which pressure air is introduced which resuspended dust settled on the bottom of the chamber. An average concentration within the exposure chamber of 4.7 mg/air in air was achieved using this system

4. Route of administration

The test substance was administered by inhalation using a polypropylene chamber hermetically sealed, except for one input and one output, operating with a slight positive pressure. The concentration within the chamber was estimated by collecting air samples using a Harvard impactor. During the study 4 dust samples were collected at the ½, 1 ½, 2 ½ and 3 ½ hours of exposure. The average concentration was 4.7 mg/L in air. 10 animals of both sexes in the trial, over a period of 4 hours at a concentration of 4.7 mg/L in air, with continuous monitoring by professionals throughout the study period. Special attention was paid to changes that may arise in fur, eyes, mucous membranes and nervous system, such as motor activity and behaviour, as well to the possible occurrence of serious clinical signs such as tremors, convulsions, salivation, diarrhoea, lethargy and coma.

5. Test substance' administration & dosage

A dose of 4.7 mg/L in air during 4 hours was administrated.

II. RESULTS AND DISCUSSION

A. Mortality

Mortality was 0% at the dose of 4.7 mg/L in air for 4 hours.

B. Toxicological signs

During the study period, there were no signs in the group of animals exposed to 4.7 mg/L in air for 4 hours of *Bacillus subtilis* IABBS03 by inhalation route during exposure to the product or during the observation period. Finally, this exposure caused the mortality of 0% of animals in the study (0/10).

C. Body weight

During the study period, there were no signs in the group of animals exposed to 4.7 mg/L in air for 4 hours of *Bacillus subtilis* IABBS03 by inhalation route during exposure to the product or during the observation period. Finally, this exposure caused the mortality of 0% of animals in the study (0/10).

D. Macroscopic pathology findings (histopathology)

The histopathological analysis revealed that the liver sample presented intense hyperemia in centrilobular zone (Z3) and portal area (Z1), with intense swelling of centrilobular hepatocytes in the centrilobular region (Z3). Lung sample showed intense congestion without inflammatory histopathology changes. Spleen showed intense hyperemia without other vascular, degenerative or inflammatory changes. Finally, the sample of kidney cortex showed moderate congestion with intense swelling of tubular epithelium without inflammatory lesions of interstitial or glomerular mesangium.

III. CONCLUSIONS

The lethal concentration 50 (LC_{50}) for this acute inhalation toxicity test of *Bacillus subtilis* IABBS03 was > 4.7 mg/L in air during 4 hours. The administration of 4.7 mg/L in air during 4 hours caused mortality of 0% of animals (0/10). Did not show symptoms associated with exposure to product *Bacillus subtilis* IABBS03 product during the exposition and the period of observation. The post- mortem macroscopic analysis of the exposed animals to test substance revealed moderate pulmonary congestion, mild petechiae in lung and moderate to severe liver congestion. The histopathological analysis revealed that the liver sample presented intense hyperemia in centrilobular zone (Z3) and portal area (Z1), with intense swelling of centrilobular hepatocytes in the centrilobular region (Z3). Lung sample showed intense congestion without inflammatory histopathology changes. Spleen showed intense hyperemia without other changes vascular, degenerative or inflammatory. Finally, the sample of kidney cortex showed moderate congestion with intense swelling of tubular epithelium without inflammatory lesions of interstitial or glomerular mesangium. The results show that CL_{50} for the product *Bacillus subtilis* IABBS03 is higher than 4.7 mg/L in air during 4 hours, these results to estimate the degree of inhalation toxicity, but this cannot be extrapolated directly to the human population. Finally this study show that CL_{50} for the substance *Bacillus subtilis* IABBS03 is higher than 4.7 mg/L in air during 4 hours.

EPA TOXICITY CATEGORY IV

DATA EVALUATION RECORD

EPA Primary Reviewer: Ibrahim S. Barsoum, Ph.D. ISB

EPA Secondary Reviewer: John L. Kough, Ph.D. JLK

Acute Dermal Irritation

Data Record	Reg. No. 89615-R Submission No. 935475 / Decision No. 478892 / DP Barcode: DP 418701 Study number IC-OCDE-PH-11/0245
Title & Author:	*Bacillus subtilis* IAB/BS/03 Technical powder. Assessment of acute dermal irritation. RICHEUX. F. (2011)
MRID No:	489693-07
Guidelines:	EPA No. 870.2500
GLP	Yes

Executive Summary

The test item *Bacillus subtilis* IAB/BS/03 Technical Powder was applied, as supplied, at the dose of 0.5 g (10^{10} CFU)/animal, under semi-occlusive dressing during 4 hours on an undamaged skin area of 3 rabbits. A slight erythema was noted on the treated area of one animal, 1 and 24 hours after the patch removal. This reaction was totally reversible on day 2.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** *Bacillus subtilis* IAB/BS/03 Technical powder
   - **Description:** White powder
   - **Lot/Batch #:** UBBS1101011
   - **Purity:** 2 x 10^{10} CFU/g
   - **Storage:** Room temperature
2. **Test animals**
   - **Species:** Albino New Zealand rabbits (3 animals)
   - **Sex:** Male
   - **Strain:** NZW
   - **Age:** 11 weeks old
   - **Weight at dosing:** 2.21-2.56 kg
   - **Source:** Elevage de Gérome (Quartier Labaste - F40260 Linxe)
   - **Diet:** Foodstuff SDS-C15, *ad libitum.*
   - **Water:** Drinking water (tap water from public distribution system), *ad libitum.*
4. **Environmental conditions**
   - **Temperature:** 17 - 23°C
   - **Humidity:** 30 - 70% relative humidity
   - **Air changes:** Minimum 15 air changes/h
   - **Photoperiod:** Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. **In life dates:** 24th May 2011 to 03rd June 2011
2. **Animal assignment and treatment**

Approximately 24 hours before the test, the back and flanks of one rabbit were shorn using electric clippers equipped with a fine comb, so as to expose an area of skin about 6 cm^2 per patch. As no tissue destruction was noted after a treatment during 1 hour and 3 minutes, the test item was applied, as supplied, at a dose of 0.5 g (10^{10} CFU /animal), on an undamaged skin area of one flank of the animal for 4 hours. The patch was secured in position with a strip of surgical adhesive tape under semi-occlusive dressing. After the removal of the patch, the treated area was rinsed with distilled water. On the opposite flank, an untreated area was served as the control. Initially, a single animal was treated. After consideration of the cutaneous responses produced in the first treated animal on day 3, two additional animals were treated during 4 hours.

3. Grading of reactions

The skin reactions were appreciated 1 hour and then 24, 48 and 72 hours after removal of the patch.

This examination consists in assessing the irritant reactions in the treated zone, compared to a control area; the following scales are used:

Grading scales:

Erythema and Eschar formation	Oedema
0 ..... No erythema	**0** ..... No oedema
1 ..... Very slight erythema (barely perceptible)	**1** ..... Very slight oedema (barely perceptible)
2 ..... Well defined erythema	**2** ..... Slight oedema (contour clearly defined)
3 ..... Moderate to severe erythema	**3** ..... Moderate oedema (raised approx. 1mm)
4 ..... Severe erythema (beef redness) with eschars formation preventing grading of erythema	**4** ..... Severe oedema (raised more than 1mm, and extending beyond area of exposure)

NOTE:
If no reaction is observed 72 hours after the treatment, the study is terminated. In case of persistent reactions, additional observations can be carried out from D4 to D14 in order to determine the reversible character of the lesions observed.

II. RESULTS AND DISCUSSION

A slight erythema was noted on the treated area of one animal, 1 and 24 hours after the patch removal. This reaction was totally reversible on day 2.

III. CONCLUSION: ACCEPTABLE. EPA TOXICITY CATEGORY IV

DATA EVALUATION RECORD

EPA Primary Reviewer: Ibrahim S. Barsoum, Ph.D. ISB

EPA Secondary Reviewer: John L. Kough, Ph.D. JLK

Acute Eye irritation

Data Record:	Reg. No. 89615-R Submission No. 935475 / Decision No. 478892 / DP Barcode: DP 418701 Study number IO-OCDE-PH-11/0245
Title:	*Bacillus subtilis* IAB/BS/03 Technical powder. Assessment of acute eye irritation.
MRID No:	489693-06
Guidelines:	EPA Guideline No. 870.2400
GLP	Yes

Executive Summary

The test item *Bacillus subtilis* IAB/BS/03 Technical Powder was instilled, as supplied, into the eye of 3 New Zealand rabbits at the dose of 0.1 g (2 x 10^9 CFU)/animal. The ocular conjunctivae reactions observed during the study have been slight to moderate and totally reversible: a slight to moderate redness noted 1 hour after the test item instillation and totally reversible between days 2 and 4, associated with a slight chemosis noted 1 hour after the test item instillation in two animals and totally reversible on day 1.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** *Bacillus subtilis* IAB/BS/03 Technical powder
   Description: White powder
   Lot/Batch #: UBBS1101011
   Purity: 2 x 10^{10} CFU/g
   Storage: Room temperature

2. **Test animals**
   Species: Albino New Zealand rabbits (3 animals)
   Sex: Male
   Strain: NZW
   Age: 12 weeks old
   Weight at dosing: 2.35-2.56 kg
   Source: Elevage de Gérome (Quartier Labaste - F40260 Linxe)
   Acclimatisation period: minimum 5 days
   Diet: Foodstuff SDS-C15, *ad libitum*.
   Water: Drinking water (tap water from public distribution system), *ad libitum*.

4. **Environmental conditions**
   Temperature: 17 - 23°C
   Humidity: 30 - 70% relative humidity
   Air changes: minimum 15 air changes/h
   Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In life dates: 30th May 2011 to 03rd June 2011

2. Animal assignment and treatment

0.1 g (2. x 10^9 CFU/animal) of the test item was instilled into the conjunctival sac of one eye after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the test item. The other eye remained untreated serving as control.

Initially, a single animal was treated. After consideration of the responses produced on day 1, two additional animals were treated.

3. Grading of reactions

Ocular examinations were performed on both right and left eyes 1 hour, 24, 48 and 72 hours following treatment, according to the numerical evaluation given below.

CHEMOSIS (A)

- No swelling 0
- Slight swelling, including the nictitating membrane 1
- Swelling with eversion of the eyelid 2
- Swelling with eyelid half-closed 3
- Swelling with eyelid more than half-closed 4

DISCHARGE (B)

- No discharge 0
- Slight discharge (normal slight secretions in the inner corner not to be taken into account) 1
- Discharge with moistening of the eyelids and neighbouring hairs 2
- Discharge with moistening of the eyelids and large areas around the eye 3

REDNESS (C)

- Blood vessels normal 0
- Vessels significantly more prominent than normal 1
- Vessels individually distinguishable with difficulty
  - Generalised red coloration 2
  - Generalised deep red coloration 3

IRIS (D)

- Normal 0
- Iris significantly more wrinkled than normal, congestion, swelling of the iris which continues to react to light, even slowly 1
- No reaction to light, haemorrhage, significant damage (any or all of these characteristics) 2

CORNEA: DEGREE OF OPACITY (E)

- No modification visible either directly or after instillation of fluorescein (no loss of glint or polish) 0
- Translucent areas (diffuse or disseminated), iris details clearly visible 1
- Easily identifiable translucent area, iris details slightly obscured 2
- Opalescent area, no iris details visible, pupil outline scarcely distinguishable 3
- Total corneal opacity, completely obscuring the iris and pupil 4

CORNEA: EXTENT OF OPACITY (F)

- Opaque area present but covering one quarter or less 1
- Between one quarter and half 2
- Between half and three quarters 3
- Between three quarters and the entire surface 4

Note:
If no reaction is observed 72 hours after instillation, the study is terminated. In case of persistent reactions, additional observations can be carried out from D4 to D21 in order to determine the reversible character of the lesions observed.

II. RESULTS AND DISCUSSION

The ocular conjunctivae reactions observed during the study have been slight to moderate and totally reversible: a slight to moderate redness noted 1 hour after the test item instillation and totally reversible between days 2 and 4, associated with a slight chemosis noted 1 hour after the test item instillation in two animals and totally reversible on day 1.

III. CONCLUSION: ACCEPTABLE. EPA TOXICITY CATEGORY IV